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Comparative Effects of Two Forage Species on Rhizosphere Acidification and Solubilization of Phosphate Rocks of Different Reactivity

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ABSTRACT

Dissolution of phosphate rocks (PR) in soils requires an adequate supply of acid (H^+) and the removal of the dissolved products [calcium (Ca^{2+}) and dihydrogen phosphate ($H_2PO_4^-$)]. Plant roots may excrete H^+ or OH^- in quantities that are stoichiometrically equal to excess cation or anion uptake in order to maintain internal electroneutrality. Extrusion of H^+ or OH^- may affect rhizosphere pH and PR dissolution. Differences in rhizosphere acidity and solubilization of three PRs were compared with triple superphosphate between a grass (*Brachiaria decumbens*) and a legume (*Stylosanthes guianensis*) forage species at two pH levels (4.9 and 5.8) in a phosphorus (P)-deficient Ultisol with low Ca content. The experiment was performed in a growth chamber with pots designed to isolate rhizosphere and non-rhizosphere soil. Assessment of P solubility with chemical extractants led to ranking the PRs investigated as either low (Monte Fresco) or high solubility (Riecito and North Carolina). Solubilization of the PRs was influenced by both forage species and mineral composition of the PR. The low solubility PR had a higher content of calcite than the high solubility PRs, which led to increased soil pH values (>7.0) and exchangeable Ca, and relatively little change in bicarbonate-extractable soil P. Rhizosphere soil pH decreased under Stylosanthes but increased under Brachiaria. The greater ability of Stylosanthes to acidify rhizosphere soil and solubilize PR relative to Brachiaria is attributed to differences between species in net ion uptake. Stylosanthes had an excess cation uptake, defined by a large Ca uptake and its dependence on N_2 fixation, which induced a significant H^+ extrusion from roots to maintain cell electroneutrality. Brachiaria had an excess of anion uptake, with nitrate (NO_3^-) comprising 92% of total anion uptake. Nitrate and sulfate (SO_4^{2-}) reduction in Brachiaria root cells may

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have generated a significant amount of cytoplasmic hydroxide (OH^-), which could have increased cytoplasmic pH and induced synthesis of organic acids and OH^- extrusion from roots.

Keywords: bicarbonate-extractable soil phosphorus, Brachiaria decumbens, exchangeable soil calcium, phosphate rock, rhizosphere acidification, Stylosanthes guianensis

INTRODUCTION

The effectiveness of phosphate rock (PR) as a fertilizer depends on the mineralogy and chemical properties of the materials and the soil, crop, environment and management factors (Khasawneh and Doll, 1978). The rate of PR dissolution is determined by the concentration of both protons (H^+) and the reaction products, calcium (Ca^{2+}) and dihydrogen phosphate ($H_2PO_4^-$), in the solution immediately surrounding the PR (Bolan and Hedley, 1990). There is general agreement among prior investigations that low pH, high exchangeable aluminum (Al), and high phosphorus (P) sorption capacity are soil properties that favor PR solubilization (Khasawneh and Doll, 1978; Smyth and Sanchez, 1982; Kanabo and Gilkes, 1988; Chien and Menon, 1995). However, these soil properties also immobilize the P dissolved from PRs and may render it less available to plants. The ideal conditions for PR dissolution and maintenance of dissolved soil P in a plant-available form would be sufficient acidity to dissolve the apatite without affecting plant growth and presence of a sink for the Ca that is released.

In order to maintain electroneutrality at the soil-root interface, plant roots excrete H^+ or hydroxide (OH^-) ions in quantities that are stoichiometrically equal to the respective excess uptake of either cations or anions (Breteler, 1973; Hedley et al., 1982). Extrusion of H^+ and OH^- affect intracellular pH; an excess of cation uptake will be associated with a corresponding net release of H^+ from roots decreasing rhizosphere pH and increasing cellular pH. The pH within the cells is maintained in the range of 7.3 to 7.6 by the operation of a so-called biochemical pH stat (Davies, 1986; Raven, 1986). Biochemical pH-stats operate when H^+ or OH^- are produced and retained within the cells. Control of intracellular pH is achieved by modification of the proportion of strong and weak organic acids via carboxylation/decarboxylation mechanisms (Davies, 1986). Production of OH^- in the cytoplasm increases cellular pH and results in activation of the enzyme phosphoenolpyruvate (PEP) carboxylase, which induces synthesis of organic acids. Whereas, formation of H^+ in the cytoplasm activates PEP decarboxylase, a carboxylic group is neutralized and the cytoplasmic pH is maintained (Haynes, 1990). Leguminous plants dependent on biological nitrogen $(N)_2$ fixation receive most of their N from N_2 fixation (reduced N form) and a sizeable excess of cation uptake could occur with a concomitant acidification of the rhizosphere and accumulation of tissue carboxylates (Israel and Jackson, 1982 and Raven et al., 1991).

Plant species growing under similar conditions may differ in their ability to absorb P from PR. Marschner (1995) stated that N_2-fixing plants have a higher capacity to utilize P from PR than nitrate-fed plants, because of their higher cation/anion uptake ratio and corresponding net release of H^+. Van Raij and Van Diest (1979) studied the utilization of P from different sources by six plant species. They concluded that the degree of usefulness of sparingly soluble phosphate sources is determined to a considerable extent by the nutritional characteristics of the crop species. Species with an alkaline-uptake pattern (high proportion of cations) were likely to make good use of PR, even when N is absorbed in nitrate (NO_3^-) form. Robinson et al. (1992) reported that there is a strong influence of Ca sink size on the dissolution of PRs. Provision of an adequate sink for Ca in soil would be expected to maintain a small concentration of Ca in solution, thereby allowing dissolution of PR to continue. The objectives of this study were to evaluate (a) the contribution of H^+ extrusion from leguminous forages on the solubilization of PRs of different reactivities, and (b) the extent to which PR dissolution and P availability to forages were influenced by soil acidity and plant nutritional status.

MATERIALS AND METHODS

Experimental Design and Management

An experiment was conducted in a growth chamber at 30/26°C day/night regime, 12/12 h light/dark period, and 30% relative humidity, using the surface 30 cm of an Ultisol (loamy siliceous, thermic Arenic Paleudults) with a pH value in water of 4.9, 3.9 mg kg^{-1} of Olsen-extractable P and 0.14 cmol of Ca kg^{-1}. The experiment consisted of a factorial combination of three factors (P source, soil pH, and forage species) arranged in a split-plot design with three replications. The forage species (Brachiaria decumbens and Stylosanthes guianensis) represented the whole-plot factor and the combination of five P sources and two soil pH levels were the subplot factors. Phosphorus treatments consisted of 50 mg of neutral ammonium acetate soluble P kg^{-1} of soil from each PR source plus a control without P. The PR sources were Monte Fresco (MFPR), Riecito (RPR), and North Carolina (NCPR). A treatment with 50 mg P kg^{-1} soil as triple superphosphate (TSP) was included as a fourth P source and a reference for soluble P. Two soil pH levels were used: the original soil pH of 4.9 and liming with 0.15 cmol Mg kg^{-1}, as magnesium carbonate ($MgCO_3$), to achieve a pH value in water of 5.8.

Pots were designed to isolate rhizosphere and non-rhizosphere soil (Figure 1). Two polyvinylchloride (PVC) tubes with a 15-cm internal diameter and 7 cm in length formed lower and upper pot sections. These sections were connected by a central compartment with two flat perforated PVC sheets containing 20 PVC tubes each with 15 mm internal diameter and 10 cm in

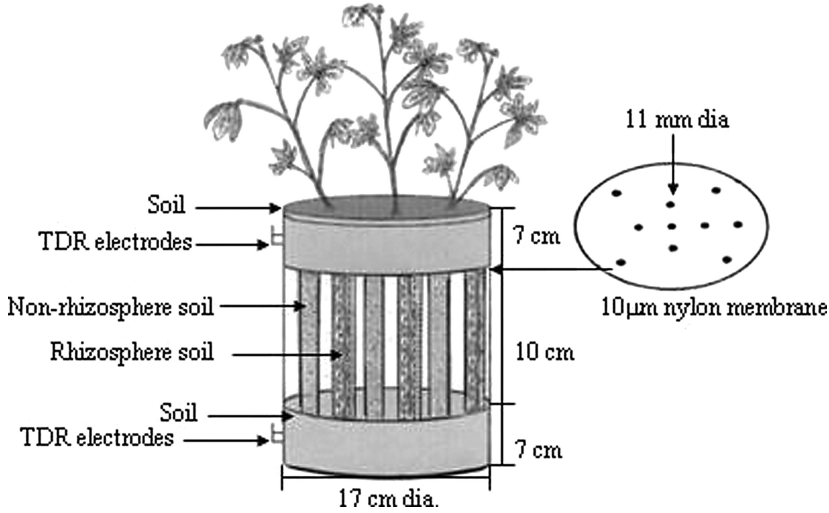

Figure 1.

length. Plastic tubes with 11 mm internal diameters were filled with soil and inserted into each of the 20 tubes. A 10 μm nylon micro filter was glued to the perforated bottom of the upper pot section and holes were cut through the filter to allow roots to extend into 10 of the 20 tubes in the middle section. This procedure allowed roots to extend through half of the soil-filled tubes into the lower compartment. Rhizosphere soil is considered soil from tubes where roots extended down to the bottom compartment. Soil from tubes where roots were blocked from entry served as reference non-rhizosphere soil. A pair of electrodes was installed horizontally in the middle of the upper and bottom compartments and soil moisture in each compartment was maintained near field capacity throughout the experiment by adding distilled water twice daily based on time domain reflectometer readings.

Stylosanthes seeds were inoculated using 4 mL/pot of an inoculum suspension (15 g of peat carrying Stylosanthes inoculum/150 mL sterile distilled water). Plants were thinned to 20 per pot for Brachiaria and 40 per pot for Stylosanthes two weeks after planting. Basal nutrients were supplied as a solution applied three times a week. Total quantities of macronutrients added to pots planted with Brachiaria during the experiment in mg kg^{-1} of soil were: 160 N , 330 potassium (K), 88 magnesium (Mg), and 150 sulfur (S) as magnesium nitrate [$Mg(NO_3)_2$], potassium nitrate (KNO_3), magnesium sulfate ($MgSO_4$), and potassium sulfate (K_2SO_4). Total quantities of micronutrients added to Brachiaria during the experiment in μg kg^{-1} of soil were: 54 manganese (Mn) as manganese sulfate ($MnSO_4 \cdot H_2O$), 4.0 copper (Cu) as copper sulfate ($CuSO_4 \cdot 5H_2O$), 6.7 boron (B) as boric acid (H_3BO_3), 2.1 molybdenum

(Mo) as sodium molybdate ($NaMoO_4 \cdot 2H_2O$), 0.36 cobalt (Co) as cobalt chloride ($CoCl_2 \cdot 6H_2O$), 364 iron (.F)e as ferric chloride ($FeCl_3 \cdot 6H_2O$), 197 sodium (Na) as disodium salt of ethylene diaminetetraacetic acid (Na_2H_2 EDTA) and sodium chloride (NaCl). Total quantities of macronutrients added to pots planted with Stylosanthes during the experiment in mg kg^{-1} of soil were 250 K, 52 Mg and 170 S as $MgSO_4$ and K_2SO_4, and the following $\mu g\ kg^{-1}$ of micronutrients: 55.6 Mn as $MnSO_4 \cdot H_2O$, 3.8 Cu as $CuSO_4 \cdot 5H_2O$, 6.9 B as H_3BO_3, 2.2 Mo as $NaMoO_4 \cdot 2H_2O$, 0.37 Co as $CoCl_2 \cdot 6H_2O$, 374.8 Fe as $FeCl_3 \cdot 6H_2O$, 216 Na as Na_2H_2 EDTA and NaCl.

Analysis of variance was performed using the Statistical Analysis System (SAS, 1992) program for a split-plot experimental design. When a significant F value was detected, least significant difference (LSD) tests were performed to separate treatment means.

Soil and PR Characterization

Total P in each PR source and P solubility in neutral ammonium citrate were determined using modifications to methods described by the Association of Official Agricultural Chemists (AOAC, 1990). Instead of a digestion in concentrated perchloric acid ($HClO_4$), each PR was ashed overnight at 500°C, dissolved in 5 mL concentrated hydrochloric acid (HCl) and filtered after dilution with distilled water. Solubility of each PR in 2% formic acid and in 2% citric acid were determined after shaking 500 mg of PR with 50 mL of each extracting solutions for 1 hr. Soluble and soil-extractable P were determined by spectrophotometry using the molybdenum blue method (Murphy and Riley, 1962). Calcium in each PR source was determined by atomic absorption in the same extract obtained for determination of total P. Soil from the upper, rhizosphere, non-rhizosphere and bottom compartments were separated at plant harvest. Soil pH was measured in a 1:2.5 soil-water ratio. Available soil P was extracted with 0.5M $NaHCO_3$ (Olsen method) in a 1:20 soil-extracting solution ratio, shaken for 30 minutes (Kuo, 1996). Changes in extracted soil P and exchangeable Ca due to PR solubilization and forage species effects were determined as the difference at the end of the experiment in the measured values between each PR treatment and the control treatment within each forage species and lime treatment. Henceforth, these differences are referred to as ΔOlsen-P and ΔCa.

Plant Analysis

Plants were harvested 8 weeks after planting. Shoots were cut at the soil surface, dried at 65°C for 3 d in a forced-draft oven, and ground for plant analysis. After digesting 1g of ground plant tissue in a solution with concentrated nitric

acid (HNO_3), 33% hydrogen peroxide (H_2O_2), and 6N HCl, Ca, Mg, K, Na, and S were determined by inductively coupled plasma and P by spectrophotometry. Total N was determined using a CHN analyzer (Perkin-Elmer PE 2400 CHN). Nitrate and ammonium (NH_4)-N were determined by colorimetry using a LACHAT Quickchem Ion Analyzer after extracting 200 mg of tissue in 10 mL hot redistilled water. Samples were shaken in a hot water bath at 85°C for 1 h and centrifuged at 3500 rpm for 20 m. Reduced-N was calculated as the difference between total-N and (NO_3-N + NH_4-N). Reduced-S was estimated as 0.05 × meq of reduced-N (Dijkshoorn and Van Wijk, 1967). Tissue SO_4^{2-} was calculated by subtracting estimated reduced-S from total S. Reduced N plus reduced S generation provides an estimate of the internal OH^-ions generated by reduction of NO_3^- and SO_4^{2-} and associated carboxylation of organic acids. Chloride was determined using a chloridometer after extracting 500 mg tissue in a mixture of 0.1 N HNO_3 and 10% acetic acid. Ash alkalinity of harvested shoots was determined by ashing overnight 500 mg of dry ground material in the presence of 0.5 meq NaOH at 550°C, dissolving the ash with 20 mL of 0.1 N HCl and titrating the excess acid to pH 5.0 with 0.1 N NaOH.

RESULTS AND DISCUSSION

Characteristics of the PR Sources

Selected chemical, mineralogical, and solubility characteristics of the PR sources used in the experiment are shown in Table 1. All PR sources were finely ground with 98% of NCPR and 96% of MFPR and RPR passing through a 0.5 mm sieve. One of the properties differentiating P release patterns among PR sources is the isomorphous substitution of carbonate for phosphate in the apatite lattice (Hammond et al., 1986). Phosphate rock solubility is conventionally estimated as the P dissolved by various extractants, because carbonate substitution is difficult to measure. According to criteria proposed by Hammond et al. (1986) our solubility values in neutral ammonium citrate place RPR and NCPR in a high solubility group and MFPR in a low solubility class. Although MFPR and NCPR have similar amounts of total Ca, they differ in free calcite ($CaCO_3$) content. The weight percentage values of apatite and total $CaCO_3$in the PRs are: 64.0 and 29.0 respectively in MFPR, 75.0 and 1.0 in RPR (Fayard and Truong, 1990) and 90.7 and 2.9 in NCPR (McClellan and Gremillion, 1980). Fayard and Truong (1990) reported that the high amount of $CaCO_3$ in MFPR makes it unsuitable to produce partially acidulated PRs, because of the $CaSO_4$ coat that forms around the PR granules during the reaction with sulfuric acid (H_2SO_4). This requires large amounts of acid during the acidulation process. Similar reaction may occur in soil, where Ca^{2+} and HCO_3^- or (CO_2+ OH^-)

Table 1
Chemical and mineralogical composition of rock phosphate materials (PR) used in the study

	PR Source		
	Monte Fresco	Riecito	North Carolina
Total P_2O_5 (%)	21.2	29.0	30.2
Ca (%)	29.1	24.8	30.1
Soluble P_2O_5(% of PR) in:			
Neutral Ammonium Citrate	1.8	8.5	8.6
2% Citric Acid	1.6	11.5	13.0
2% Formic Acid	2.3	9.9	19.9

would be the first reaction products from MFPR dissolution, increasing soil pH and damping MFPR dissolution.

Effects of Forage Species and P Source on Rhizosphere Soil pH

The soil pH in the rhizosphere tubes at harvest of the experiment was significantly influenced by the main effects and interactions between forage species, lime and P treatments (Table 2). The liming effect of the MFPR source was evident with both forage species; soil pH values in the rhizosphere tubes for both species were in the range of 7.3–7.4 without lime and 7.3–7.6 with lime. Mean values of soil pH averaged across lime and P treatments indicate a general trend for greater soil acidification in pots with the forage legume than with the grass. The mean soil pH difference between rhizosphere and non-rhizosphere tubes increased by 0.12 units in pots with Brachiaria and decreased by 0.58 units in pots with Stylosanthes. The pH difference between rhizosphere and non-rhizosphere soil was influenced by both forage species and P treatment. With the MFPR source pH differences were small for both species. Among the other P treatments rhizosphere soil for Stylosanthes was acidified, relative to non-rhizosphere, in the order of TSP $>$ RPR $\approx$ NCPR $>$ control. In pots with Brachiaria, however, pH of the rhizosphere soil was greater than for non-rhizosphere soil in the control, RPR, and NCPR treatments.

Effect of Forage Species on PR Dissolution

The dissolution of PR can be assessed by measuring the residual, undissolved PR or the amounts of P and/or Ca present in the soil and plants (Bolan and Hedley, 1990). The amount of Ca and P released from PRs are denoted by ΔCa and ΔOlsen-P. They represent the difference in amount of soil exchangeable

Table 2
Mean values of soil pH in rhizosphere (R) and non-rhizosphere (NR) tubes and the difference between rhizosphere and non-rhizosphere (R – NR) tubes, Δ Ca and Δ Olsen-P in rhizosphere soil after harvest as a function of forage species, lime and P treatments

	Treatment		pH in Water				
Species	pH	P	R	NR	R-NR	ΔCa	ΔOlsen-P
						$cmol_c kg^{-1}$	mg kg^{-1}
Brachiaria	4.9	Control	5.47	5.18	0.29	—	—
		MFPR	7.39	7.46	−0.07	1.22	−0.72
		RPR	5.47	5.53	−0.06	0.38	7.20
		NCPR	5.83	5.67	0.16	0.25	5.32
		TSP	5.28	5.40	−0.12	0.23	22.40
	5.8	Control	6.10	5.75	0.35	—	—
		MFPR	7.56	7.51	0.04	1.21	0.10
		RPR	6.6	6.13	0.47	0.13	2.97
		NCPR	6.27	6.06	0.21	0.35	2.54
		TSP	5.69	5.79	−0.10	0.25	22.11
Mean for Brachiaria			6.17	6.05	0.12	0.50	7.74
Stylosanthes	4.9	Control	4.74	4.82	−0.08	—	—
		MFPR	7.34	7.30	0.03	1.01	−1.09
		RPR	4.44	5.22	−0.78	0.59	13.63
		NCPR	4.76	5.29	−0.53	0.68	12.24
		TSP	3.99	4.87	−0.88	0.06	25.53
	5.8	Control	4.68	5.26	−0.58	—	—
		MFPR	7.26	7.25	0.01	1.08	−0.82
		RPR	4.63	5.48	−0.85	0.48	8.59
		NCPR	4.67	5.64	−0.96	0.54	11.56
		TSP	4.13	5.31	−1.18	0.24	29.45
Mean for Stylosanthes			5.06	5.64	−0.58	0.59	12.39
					pH Treatment Means		
	4.9		5.47	5.67	−0.2	0.55	10.56
	5.8		5.76	6.02	−0.26	0.53	9.56
					P Treatment Means		
		Control	5.25	5.25	0.00		
		MFPR	7.39	7.38	0.01	1.13	−0.63
		RPR	5.29	5.59	−0.30	0.39	8.1
		NCPR	5.38	5.66	−0.28	0.45	7.91
		TSP	4.77	5.34	−0.57	0.19	24.87
$LSD_{0.05}$							
Species			0.09	0.07	0.10	NS¶	1.21
pH			0.09	0.07	NS	NS	NS
Species × pH			0.13	0.10	0.14	NS	NS
P			0.14	0.11	0.15	0.15	1.72
P × Species			0.20	0.15	0.21	0.21	2.42
P × pH			0.20	0.15	0.21	NS	2.42
Species × P × pH			NS	NS	NS	NS	NS

¶NS = F value for effect not significant at $p \leq 0.05$.

Ca and Olsen-P between P source treatments and the control treatment without P at the end of the experiment.

There are significant differences ($P < 0.05$) in ΔCa in rhizosphere soil among P treatments and species (Table 2). The large ΔCa value for MFPR treatments under both forage species is associated with increases in soil pH to values > 7.0 and can be attributed to the Ca released from $CaCO_3$ in this source. The application of 50 mg of neutral ammonium acetate soluble P kg^{-1} of soil with each PR source led to the supply of different amounts of Ca due to variations in their chemical and mineralogical composition (Table 1). Amounts of Ca added with each P source were 1870 for MFPR, 336 for RPR, 408 for NCPR and 32 mg kg^{-1} for TSP. There was no significant difference in ΔCa values between forage species when averaged across lime and P treatments, but the average amounts of Ca released from NCPR and RPR were larger in Stylosanthes than in Brachiaria. The ΔCa soil data among the three PR sources are in agreement with the results for soil rhizosphere acidification (Table 2), shoot dry weight (Table 3) and Ca uptake (Table 4) among the forage species and the three PR sources. Higher acidity in the rhizosphere soil of Stylosanthes with RPR and NCPR would favor greater PR dissolution, plant growth and Ca uptake than with MFPR or Brachiaria.

Differences in rhizosphere ΔOlsen-P values among P sources were significantly affected by forage species and lime treatments (Table 2). Mean values of ΔOlsen-P in rhizosphere soil averaged across P source and lime treatments were larger for Stylosanthes than for Brachiaria. Values of ΔOlsen-P in rhizosphere soil among P sources, when averaged across forage species and lime treatments, had the following order: TSP $>$ RPR $\approx$ NCPR $>$ MFPR. Maximum ΔOlsen-P values for TSP treatments in pots with both forage species is consistent with the high solubility of P in this source relative to that of the PR sources. Minimum ΔOlsen-P values for MFPR among all P sources is also consistent with this material's low solubility in neutral ammonium citrate, formic acid and citric acid (Table 1) and the limited PR dissolution which would occur when rhizosphere soil pH is > 7.0 (Table 2). Likewise, higher ΔOlsen-P values for RPR and NCPR with Stylosanthes than with Brachiaria are consistent with greater acidification of the rhizosphere by the forage legume, which would favor more PR dissolution and P release than in the rhizosphere of a forage grass. The higher values for P release to rhizosphere soil (ΔOlsen-P) with RPR than NCPR in unlimed soil, when averaged across forage species, and the lower rhizosphere soil pH values with RPR than NCPR in unlimed soil with Brachiaria and Stylosanthes (Table 2) indicate that RPR performs better than NCPR as soil acidity increases.

Dry Matter Yield

There were significant differences ($P < 0.05$) in shoot dry weight between forage species, P treatments and the interaction between forage species and P

Table 3
Mean values of shoot dry weight, nodule fresh weight and total N uptake in Brachiaria and Stylosanthes as a function of lime and P treatments

Treatment			Shoot	Nodule	Total N
Species	pH	P	Dry weight	Fresh Weight	Uptake
			g/pot	mg/pot	
Brachiaria	4.9	Control	2.36	—	80.9
		MFPR	1.74	—	65.9
		RPR	20.99	—	444.8
		NCPR	20.05	—	412.6
		TSP	24.69	—	467
	5.8	Control	1.62	—	62.9
		MFPR	1.60	—	62.4
		RPR	14.71	—	359.1
		NCPR	19.53	—	421.8
		TSP	23.71	—	454.4
Mean for Brachiaria			13.10	—	283.2
Stylosanthes	4.9	Control	2.28	0.22	48.1
		MFPR	1.00	0.06	16.1
		RPR	9.17	0.73	254.0
		NCPR	8.92	0.71	241.4
		TSP	9.52	0.90	251.1
	5.8	Control	3.06	0.27	67.5
		MFPR	1.70	0.14	31.7
		RPR	8.63	0.76	223.4
		NCPR	9.41	0.89	265.8
		TSP	9.83	0.88	270.5
Mean for Stylosanthes			6.35	0.56	167.4
			pH Treatment Means		
	4.9		10.07	0.52	228.6
	5.8		9.38	0.59	222.0
			P Treatment Means		
		Control	2.33	0.24	64.8
		MFPR	1.51	0.1	44.0
		RPR	13.38	0.75	321.4
		NCPR	14.48	0.80	335.4
		TSP	16.94	0.89	360.8
$LSD_{0.05}$					
Species			0.84	—	22.8
pH			NS¶	NS	NS
Species x pH			NS	—	NS
P			1.13	0.19	36.1
P × Species			1.88	—	51.0
P × pH			1.88	NS	NS
Species × P × pH			NS	—	NS

¶ = F value for effect is not significant at $p \leq 0.05$.

Table 4

Mean values of cation uptake, anion uptake, sum of cation (ΣC_u) and anion (ΣA_u) uptake, excess cation uptake (ΣC_u - ΣA_u) and cation/anion uptake ratio ($\Sigma C_u/\Sigma A_u$) in Brachiaria and Stylosanthes as a function of lime and P treatments

Treatment			Cation uptake				Anion uptake				Ion uptake		Excess cation uptake	Cation/Anion uptake ratio
Species	pH	P	Ca	Mg	K	Na	$H_2PO_4^{-1}$	SO_4^{-2}	NO_3^{-1}	Cl^{-1}	ΣC_u	ΣA_u	$\Sigma C_u - \Sigma A_u$¶	$\Sigma C_u/\Sigma A_u$
								me/pot						
Brachiaria	4.9	Control	0.14	0.92	2.74	0.01	0.04	0.24	5.78	0.13	3.81	6.2	−2.38	0.62
		MFPR	0.37	0.75	2.14	0.02	0.03	0.22	4.71	0.09	3.28	5.04	−1.76	0.65
		RPR	1.33	5.46	19.80	0.03	0.67	1.52	31.76	0.21	26.62	34.16	−7.55	0.78
		NCPR	1.20	4.79	17.87	0.02	0.63	1.65	29.51	0.20	23.88	31.99	−8.11	0.75
		TSP	1.15	5.68	21.33	0.03	0.94	1.66	33.43	0.20	28.19	36.22	−8.03	0.78
	5.8	Control	0.10	0.97	2.15	0.02	0.03	0.25	4.49	0.10	3.24	4.86	−1.62	0.67
		MFPR	0.38	0.90	2.06	0.02	0.02	0.33	4.46	0.11	3.36	4.93	−1.56	0.68
		RPR	0.74	4.85	14.27	0.02	0.37	1.21	25.66	0.24	19.88	27.48	−7.6	0.73
		NCPR	1.17	5.62	16.54	0.03	0.49	1.63	30.10	0.20	23.35	32.41	−9.06	0.72
		TSP	1.18	7.03	20.40	0.02	1.02	1.83	32.48	0.24	28.63	35.57	−6.93	0.80
Mean for Brachiaria			0.78	3.70	11.93	0.02	0.42	1.05	20.24	0.17	16.42	21.89	−5.46	0.72
Stylosanthes	4.9	Control	1.46	0.63	1.53	0.00	0.07	0.46	0.00	0.06	3.62	0.59	3.03	6.27
		MFRP	0.92	0.21	0.69	0.00	0.02	0.35	0.00	0.02	1.82	0.40	1.42	4.59
Stylosanthes		RPR	6.39	2.42	6.87	0.03	0.68	1.58	0.00	0.29	15.71	2.55	13.16	6.07
		NCPR	6.73	2.12	6.57	0.03	0.62	1.65	0.00	0.25	15.46	2.52	12.94	5.94
		TSP	4.48	2.99	7.07	0.04	0.60	2.10	0.00	0.20	14.58	2.90	11.68	5.11
	5.8	Control	1.76	0.91	2.21	0.01	0.09	0.48	0.00	0.13	4.89	0.70	4.20	7.49
		MFPR	1.59	0.39	1.09	0.01	0.05	0.43	0.00	0.05	3.08	0.53	2.55	5.84

(Continued on next page)

Table 4
Mean values of cation uptake, anion uptake, sum of cation (ΣC_u) and anion (ΣA_u) uptake, excess cation uptake ($\Sigma C_u - \Sigma A_u$) and cation/anion uptake ratio ($\Sigma C_u / \Sigma A_u$) in Brachiaria and Stylosanthes as a function of lime and P treatments *(Continued)*

Treatment			Cation Uptake				Anion Uptake				Ion Uptake		Excess Cation Uptake	Cation/Anion Uptake Ratio
Species	pH	P	Ca	Mg	K	Na	$H_2PO_4^{-1}$	SO_4^{-2}	NO_3^{-1}	Cl^{-1}	ΣC_u	ΣA_u	$\Sigma C_u - \Sigma A_u$¶	$\Sigma C_u / \Sigma A_u$
		RPR	5.65	2.64	6.32	0.03	0.56	1.39	0.00	0.28	14.64	2.23	12.41	6.56
		NCPR	6.54	2.72	6.90	0.03	0.66	1.58	0.00	0.28	16.18	2.53	13.65	6.41
		TSP	4.96	3.34	7.31	0.03	0.67	1.98	0.00	0.33	15.64	2.98	12.66	5.26
Mean for Stylosanthes			4.05	1.84	4.66	0.02	0.40	1.20	0.00	0.19	10.56	1.79	8.77	5.95
								pH Treatment Means						
	4.9		2.42	2.60	8.66	0.02	0.43	1.14	10.52	0.17	13.70	12.26	1.44	3.15
	5.8		2.41	2.94	7.93	0.02	0.40	1.11	9.72	0.20	13.29	11.42	1.87	3.52
								P Treatment Means						
		Control	0.86	0.86	2.16	0.01	0.06	0.36	2.57	0.10	3.89	3.09	0.81	3.76
		MFPR	0.82	0.56	1.50	0.01	0.03	0.33	2.29	0.07	2.89	2.72	0.16	2.94
		RPR	3.53	3.84	11.81	0.03	0.57	1.43	14.35	0.26	19.21	16.61	2.61	3.53
		NCPR	3.91	3.81	11.97	0.03	0.60	1.63	14.90	0.23	19.72	17.36	2.36	3.45
		TSP	2.94	4.76	14.03	0.03	0.81	1.89	16.48	0.24	21.76	19.42	2.35	2.99
$LSD_{0.05}$														
Species			0.49	0.23	0.60	NS$^{\delta}$	NS	NS	0.73	NS	1.20	0.78	1.03	0.37
pH			NS	0.23	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Species × pH			NS	0.32	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P			0.78	0.36	0.94	0.01	0.09	0.09	1.15	0.05	1.90	1.23	1.63	0.59
P × Species			1.10	0.51	1.33	0.01	0.12	0.12	1.63	0.08	2.69	1.74	2.31	0.83
P × pH			NS	0.51	1.34	NS	0.12	NS	NS	NS	NS	NS	NS	NS
Species × P × pH			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

¶Negative values mean excess anion uptake and excretion of OH^- from roots.

§Values less than 1.0 mean excess anion uptake and excretion of OH^- from plant roots.

ΔNS = F value for effect is not significant at $p \leq 0.05$.

treatments. Mean shoot dry weight of Brachiaria was twice that of Stylosanthes (Table 3). Shoot dry weight of both species increased with solubility of the P source when averaged across lime treatments. There was no significant difference in shoot dry weight for Stylosanthes between NCPR, RPR, and TSP treatments or between MFPR and the control treatment. Liming had no significant effect on shoot dry weight within or across forage species (Table 3). However, there was an appreciable reduction in shoot dry weight for Brachiaria with increasing soil pH levels under the RPR treatment. Liming apparently decreased RPR dissolution under Brachiaria.

Effect of P Source on Nodulation and N fixation by Stylosanthes

There were significant differences ($P < 0.05$) in nodule fresh weight and total N uptake in Stylosanthes between P treatments (Table 3). Mean nodule fresh weight and total N uptake in Stylosanthes, averaged across lime treatments, increased with solubility of the P source. Nodule fresh weight and total N uptake for RPR, NCPR, and TSP treatments were significantly different from values for MFPR and the control treatment. There was a linear relation ($r = 0.99$) between nodule fresh weight and total N uptake in Stylosanthes (Figure 2). The more soluble forms of PR increased shoot N concentrations and shoot dry mass of Stylosanthes compared to the control and the low solubility MFPR (Table 3). Shoot N concentrations, averaged across pH treatments, were 1.75% for MFPR, 2.70 for RPR, and 2.75 for NCPR. A similar response was observed for N_2-

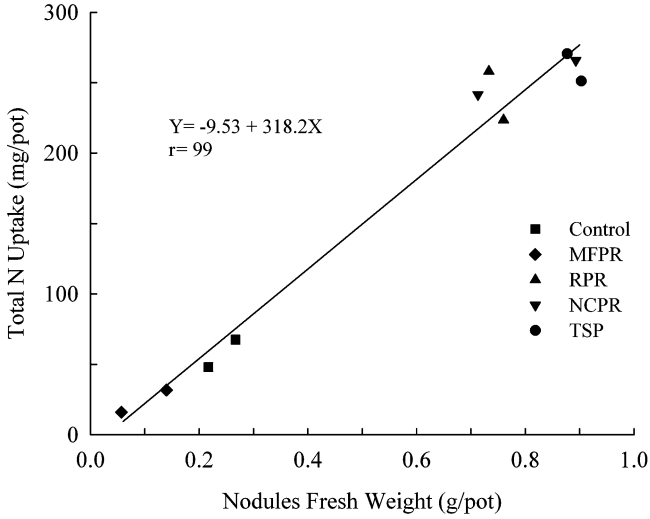

Figure 2.

fixing soybean plants as the external P supply was increased from deficient to sufficient levels (Israel, 1987). These relationships and the red internal color of nodules indicated effective nodulation and a high dependence of Stylosanthes growth on symbiotic N_2 fixation.

Plant Ion Uptake

Forage species differed in their ion uptake patterns. There were significant differences ($P < 0.05$) in Ca, Mg, K, and NO_3-N uptake between species when averaged across lime and P treatments (Table 4). The amount of ion uptake for Brachiaria was in the order of NO_3-N > K > Mg > S> Ca > P > Cl > Na. The order of ion uptake for Stylosanthes was K > Ca > Mg > S > P > Cl > Na > NO_3-N. For Brachiaria, mean NO_3^- uptake averaged across P and lime treatments comprised 92% of total anion uptake. Most of the N entered Stylosanthes in the reduced form (fixed N_2), and Ca and K comprised 82% of total cation uptake.

Cation-Anion Balance

The model proposed by Israel and Jackson (1978) was used to account for changes in rhizosphere acidity in response to differential uptake rates of cations and anions and the regulation of cation-anion balance and cytoplasmic acidity in plant tissue. The model assumes that the dominant process responsible for the root plasmalemma electrical potential is ATPase activity; total cation and total anion (organic and inorganic) charges of plant tissue must be equal and the pH of the cell cytoplasm must be maintained between 7 and 8. The quantities of OH^- and H^+ excreted are stoichiometrically equal to the respective excess of cation or anion uptake, and transport of cations, organic and inorganic anions, and organic N forms into and out of the xylem and vacuoles also must occur in a manner that allows for maintenance of charge balance (Israel and Jackson, 1982). Cations may enter the root plasma membrane in response to an electrical potential gradient. The cytoplasmic OH^- generated as the result of the H^+ extrusion process serve as counter ions for anion uptake. Nitrate and SO_4^{2-} reduction in root cells provide additional OH^- ions, which may support anion uptake or increase cytoplasmic pH. Increase in cytoplasmic pH stimulates PEP carboxylase activity and synthesis of organic acids. Synthesis or decarboxylation of organic acids may occur in order to regulate cellular pH. Decarboxylation of organic acids provides a source of OH^- ions for sustaining anion uptake in excess of cation uptake.

Data from the present study revealed significant differences ($P < 0.05$) in total cation (ΣC_u) and anion (ΣA_u) uptake, excess cation uptake ($\Sigma C_u - \Sigma A_u$) and the cation/anion uptake ratio ($\Sigma C_u / \Sigma A_u$) between forage species, P

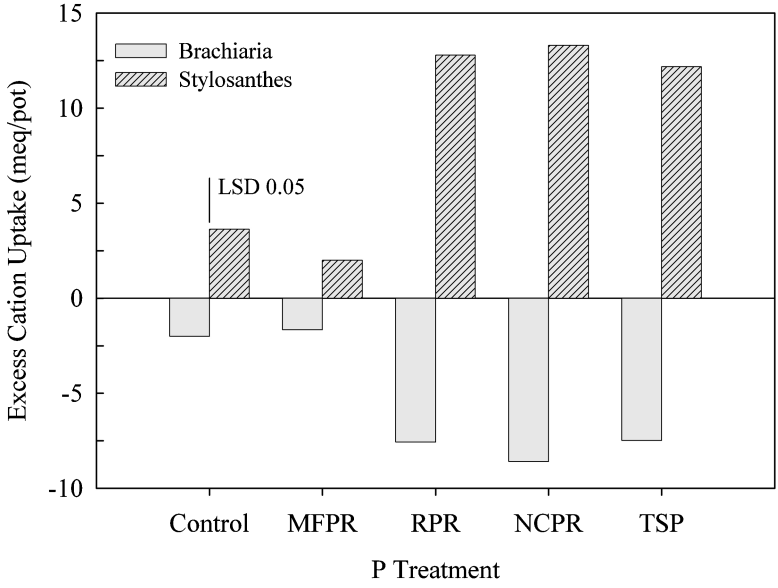

Figure 3.

treatments and the interaction between forage species and P treatments (Table 4). For Stylosanthes cation uptake exceeded anion uptake ($\Sigma C_u - \Sigma A_u$) among P treatments by values ranging from 2 to 13.3 meq/pot, whereas, anion uptake exceeded cation uptake (negative values) for Brachiaria by values ranging from -1.7 to -8.6 meq/pot (Figure 3). There were significant differences in excess cation or anion uptake between P sources with low (MFPR) and high solubility (RPR, NCPR and TSP), but there were no significant differences within PR solubility classes with either forage species. Differential changes in soil pH under both forage species for the control, RPR, NCPR, and TSP treatments were observed (Figure 4). There was a difference of 1.1 pH units in the control treatment between Brachiaria and Stylosanthes when averaged across lime treatments. Soil pH decreased from control treatment values with increasing shoot growth and excess cation uptake in Stylosanthes and the opposite occurred with increasing shoot growth and excess anion uptake (negative values) in Brachiaria. Stylosanthes and Brachiaria accumulated similar amounts of P in shoots when soil was amended with soluble PR sources (Table 4). At the end of the experiment Olsen P in rhizosphere soil of Stylosanthes was approximately double that in rhizosphere soil of Brachiaria (Table 2). Collectively, these observations support the conclusion that rhizosphere acidification associated with excess cation uptake by Stylosanthes enhanced solubilization of RPR and NCPR.

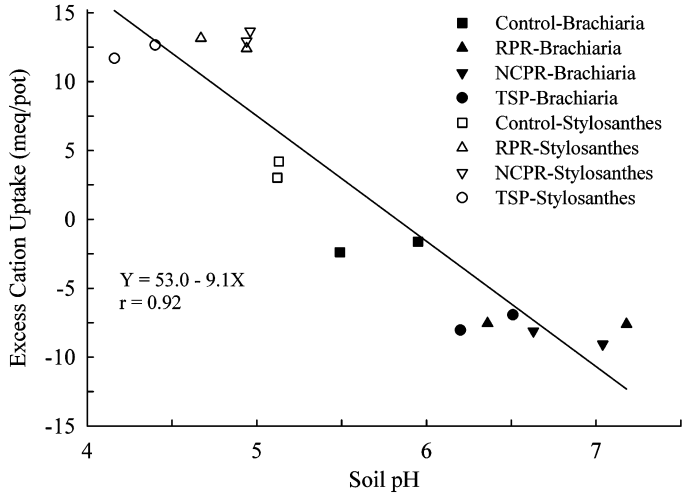

Figure 4.

There was a significant linear relation across all soil treatments and forage species between shoot ash alkalinity and excess internal cation accumulation (Figure 5). Jarvis and Robson (1983) reported a similar relationship between excess cation accumulation and ash alkalinity in subterranean clover tissue. Thus, ash alkalinity is an alternative and more convenient method for assessing

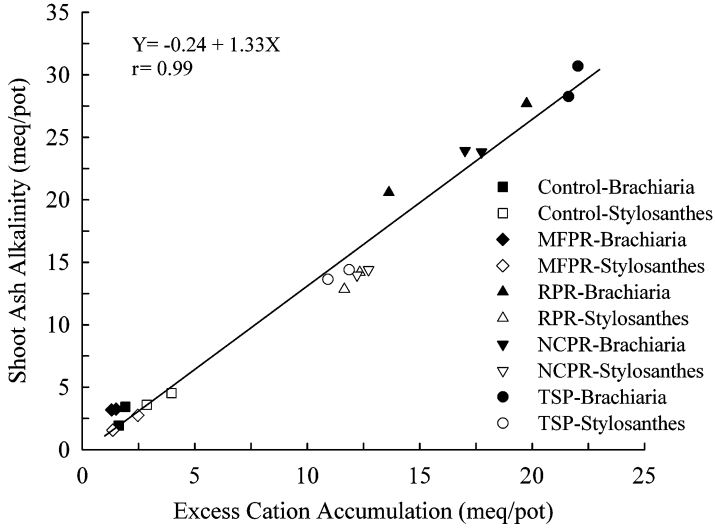

Figure 5.

internal ion balance of plants and the consequent changes in rhizosphere pH in future investigations.

Mean cation/anion uptake ratio averaged across P and lime treatments for Stylosanthes exceeded the value for Brachiaria by 8 fold (Table 4). The difference in cation/anion uptake ratio between forage species is also in agreement with observed differences in soil pH. Cation/anion ratio values < 1.0 indicate an excess of anion uptake and increasing soil pH through net released of OH^-, whereas positive cation/anion ratios corresponded with an excess of cation uptake and decreasing soil pH through release of H^+.

CONCLUSIONS

Despite adding equal amounts of P (50 mg kg^{-1} soil) soluble in neutral ammonium acetate from each PR source, results from this study showed that solubilization of the PRs was influenced by both forage species and mineral composition of the PR. The low solubility Monte Fresco PR had a higher content of calcite than the high solubility PRs (Riecito and North Carolina), which led to increased values of soil pH (>7.0) and exchangeable Ca, and reduced $\geq$Olsen-P values and P uptake from the MFPR treatment. Stylosanthes had an excess of cation uptake, defined by a large Ca uptake and a dependence on N_2 fixation for its N supply. Collectively, these factors led to a significant H^+ extrusion from Stylosanthes roots to maintain cell electroneutrality. Brachiaria had an excess of anion uptake, with NO_3^- comprising 92% of total anion uptake. Nitrate and SO_4^{2-} reduction in Brachiaria root cells may have generated a significant amount of cytoplasmic OH^-, which could have increased cytoplasmic pH and induced synthesis of organic acids and OH^- extrusion from roots. Differences in H^+ and OH^- extrusion between forage species were reflected in the soil as a decrease in rhizosphere pH and greater ΔOlsen-P values under Stylosanthes relative to Brachiaria, and an increase in rhizosphere pH under Brachiaria.

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